

Influence of dormancy regulating compounds and salinity on the germination of *Zygophyllum simplex* L. seeds

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(Accepted November 2001)

Summary

The role of dormancy regulating compounds (ethephon, fusicoccin, nitrate and thiourea) in alleviating the inhibitory effects of salinity on seed germination of an annual forb, *Zygophyllum simplex*, was studied. In the non-saline controls only 60% of the seeds germinated and germination decreased with an increase in salinity, with no seeds germinating at 125 mM NaCl. All dormancy regulating compounds significantly promoted germination in the non-saline control. Nitrate and thiourea had some promotive effect at 25 mM NaCl, but no stimulation of germination was detected at either 75 or 125 mM NaCl. Ethylene application significantly promoted germination at all salinities. Fusicoccin completely reversed the inhibitory effects of all salinity treatments on the germination of *Z. simplex*.

Introduction

Zygophyllum simplex is commonly found on saline flats around Karachi, Pakistan, with seeds germinating after heavy monsoon rains during July or August. Seeds of *Z. simplex* are moderately tolerant to salinity during germination, but no seeds germinated at 175 mM NaCl (Khan and Ungar, unpublished data). Cool and warm temperature regimes inhibited germination and greatest germination was obtained at a moderate temperature regime (15:25°C). High salinity in the medium caused high seed mortality and only 20% recovery was recorded in the best germination conditions (Khan and Ungar, 1997). Proline and betaine alleviated the innate dormancy of *Z. simplex* seeds (Khan and Ungar, 1997) and salt induced dormancy at very low salinities but at higher salinities these compatible osmotica did not promote germination. GA and kinetin substantially alleviated both innate and salinity induced dormancy in *Z. simplex* seeds (Khan and Ungar, 1997).

Fusicoccin, a diterpene glycoside, was shown to stimulate cell enlargement (Lado *et al.*, 1974) and the site of action might be the cell membrane where it activates the release of protons and hence stimulates the cell-wall loosening process (Galli *et al.*, 1980). It promoted germination of lettuce seeds inhibited by ABA and overcame the inhibitory effects of far-red light on the germination of maize and radish seeds in a similar manner as kinetin (Lado *et al.*, 1974). ABA inhibited proton extrusion and K⁺ uptake during the germination of seeds

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and this response was reversed by fusicoccin (Ballarin-Denti and Cocucci, 1979; Galli *et al.*, 1980). Ballarin-Denti and Cocucci (1980) observed a transient rise in the transmembrane potential in the cortical cells of germinating *R. sativus* seeds, which was inhibited by ABA and stimulated by fusicoccin. They suggested that the rise in transmembrane potential could be due to proton extrusion, while the subsequent reduction in potential difference resulted from the uptake of potassium. Fusicoccin might antagonize the inhibitory effect of ABA on proton extrusion but not the potassium import. Galli *et al.* (1980) reported that fusicoccin reversed the inhibition of fresh weight increase caused by treatment of *Halopappus gracilis* seeds with ABA during the early stage of germination. A treatment of fusicoccin was as effective as a combination of kinetin, ethylene and GA in alleviating stress, suggesting that fusicoccin might affect processes influenced by both cytokinins and gibberellins (Braun and Khan, 1976).

Ethylene is known to stimulate germination of seeds that may or may not be dormant (Corbineau and Come, 1995). It is effective in breaking primary seed dormancy in subterranean clover (Esashi and Leopold, 1969) and *Rumex crispus* L. (Taylorson, 1979) and embryo dormancy of apple (Sinska and Gladon, 1984) and sunflower seeds (Corbineau *et al.*, 1990). In contrast, ethylene inhibited germination in *Potentilla norvegica* L. seeds (Suzuki and Taylorson 1981). Ethylene is also known to interact with many other factors, stimulating germination in combination with increased oxygen tension (Esashi *et al.*, 1987). It allows dormant sunflower seeds to germinate in hypoxia (Corbineau and Come, 1992) and permits cocklebur seeds to germinate under water-stress conditions (Esashi *et al.*, 1989).

Nitrate has received considerable attention as a possible regulator of seed germination (Egley, 1995). The nitrate ions are reported to enhance germination, usually when combined with other factors such as alternating temperatures, chilling, light, or plant growth regulators (Bewley and Black, 1982). Thiourea counteracted the inhibitory effect of ABA and decreased the level of cytokinins in plant tissues (Esashi *et al.*, 1979). These adverse hormonal changes occurred when plant tissues were subjected to water stress (Kabar and Baltepe, 1989). Thiourea was highly effective in alleviating the inhibition of germination due to salt or high temperature stress (Esashi *et al.*, 1979; Noor and Khan, 1995).

The effect of germination regulating substances, such as nitrate, thiourea, ethephon and fusicoccin, was investigated to determine whether or not they would be effective in alleviating either an innate dormancy or the salt induced inhibition of germination in *Zygophyllum simplex* seeds.

Materials and methods

Zygophyllum simplex L. is a succulent annual in the family Chenopodiaceae, which is widely distributed in the dry subtropical deserts of Asia and Africa (Schmida, 1985). Stewart (1972) reported that its distribution ranges from the coastal areas of Sindh and Balochistan to the plains of Punjab in Pakistan. Seeds of *Zygophyllum simplex* were collected during the fall 1994 from salt flats situated on the Karachi University campus, Pakistan. Seeds were separated from the inflorescence and stored at 4°C. These seeds were brought to Ohio University, USA and germination studies were started in April 1996. Seeds were surface

sterilized using the fungicide Phygon. Germination was carried out in 50 × 9-mm (Gelman No. 7232) tight-fitting plastic Petri dishes with 5 ml of test solution. Each dish was placed in a 10-cm-diameter plastic Petri dish as an added precaution against loss of water by evaporation. Four replicates of 25 seeds each were used for each treatment. The emergence of the radicle from seeds was used as an indication of germination.

Seeds were germinated in a growth chamber at an alternating temperature regime of 15:25°C, where the higher temperature coincided with the 12-hr light period (Sylvania cool white fluorescent lamps, 25 μmol m⁻² s⁻¹, 400 - 700 nm) and the lower temperature coincided with the 12-hr dark period. Seeds were treated with germination promoting substances containing either nitrate (20 mM), thiourea (10 mM), ethephon (10 mM), or fusicoccin (5 μM) and salt solutions containing 0, 25, 75 and 125 mM NaCl. Percent germination was recorded every alternate day for 20 days. The rate of germination was estimated by using a modified Timson index of germination velocity = $\Sigma G/t$, where G is the percent seed germination at 2-days intervals and t is the total germination period (Khan and Ungar, 1984). The maximum germination velocity value possible using this index with our data was 50 (i.e., 1000/20).

Germination data were transformed (arcsine) before statistical analysis. An ANOVA analysis was used to determine if significant differences were present among means. A Bonferroni test was carried out to determine if significant ($P < 0.05$) differences occurred between individual treatments (SPSS, 1996).

Results

Seed germination of *Zygophyllum simplex* was inhibited with an increase in salinity and no germination was recorded in controls at 125 mM NaCl (figure 1). Only 60% of the seeds germinated in the distilled water control. Inclusion of ethephon, fusicoccin, nitrate and urea significantly ($P < 0.001$) promoted germination in the 0 and 25 mM NaCl in comparison to the untreated control (figure 1). Nitrate and thiourea were ineffective in promoting germination in the 75 and 125 mM NaCl treatments (figure 1). Ethephon partially increased germination at all salinities. At 125 mM NaCl, 30% germination was obtained in seeds treated with ethephon in comparison to 0% in the untreated control. However, fusicoccin completely overcame the inhibitory effect of salinity on the germination of *Z. simplex* seeds. At 125 mM NaCl, fusicoccin stimulated 80% germination as compared to 0% in the untreated seeds. All of the growth regulator treatments significantly increased germination in comparison with the control at 0 and 25 mM NaCl (figure 1). However, at higher salinities only ethephon and fusicoccin alleviated the effects of salinity and the latter completely reversed the inhibitory effect of salinity in all treatments (figure 1). A two way ANOVA indicated a significant ($P < 0.0001$) main effect of both salinity and dormancy relieving compounds and their interaction on seed germination (table 1). A one-way ANOVA for each dormancy regulating compound revealed that seed germination was significantly increased at different NaCl concentrations by thiourea, $F = 54.5$, $P < 0.00001$; ethephon, $F = 18.0$, $P < 0.0001$, nitrate, $F = 140.0$, $P < 0.0001$; fusicoccin, $F = 9.2$, $P < 0.0019$.

The velocity of germination in the 0 and 25 mM NaCl treatment was significantly enhanced by all dormancy alleviating chemicals (figure 2). Nitrogen and thiourea were

ineffective in stimulating the germination rate at higher salinities. Ethephon partially enhanced the rate of germination and fusicoccin substantially increased the rate of germination at higher salinities. A two way ANOVA of percent germination indicated a significant ($P < 0.0001$) main effect of salinity and dormancy regulating compounds and their interaction on germination velocity (table 1). A one-way ANOVA of germination velocity for each dormancy relieving compound revealed that at 0 mM NaCl velocity is significantly increased by thiourea, $F = 63.5$, $P < 0.00001$; ethephon, $F = 35.1$, $P < 0.0001$, nitrate, $F = 133.8$, $P < 0.0001$; fusicoccin, $F = 18.9$, $P < 0.0001$.

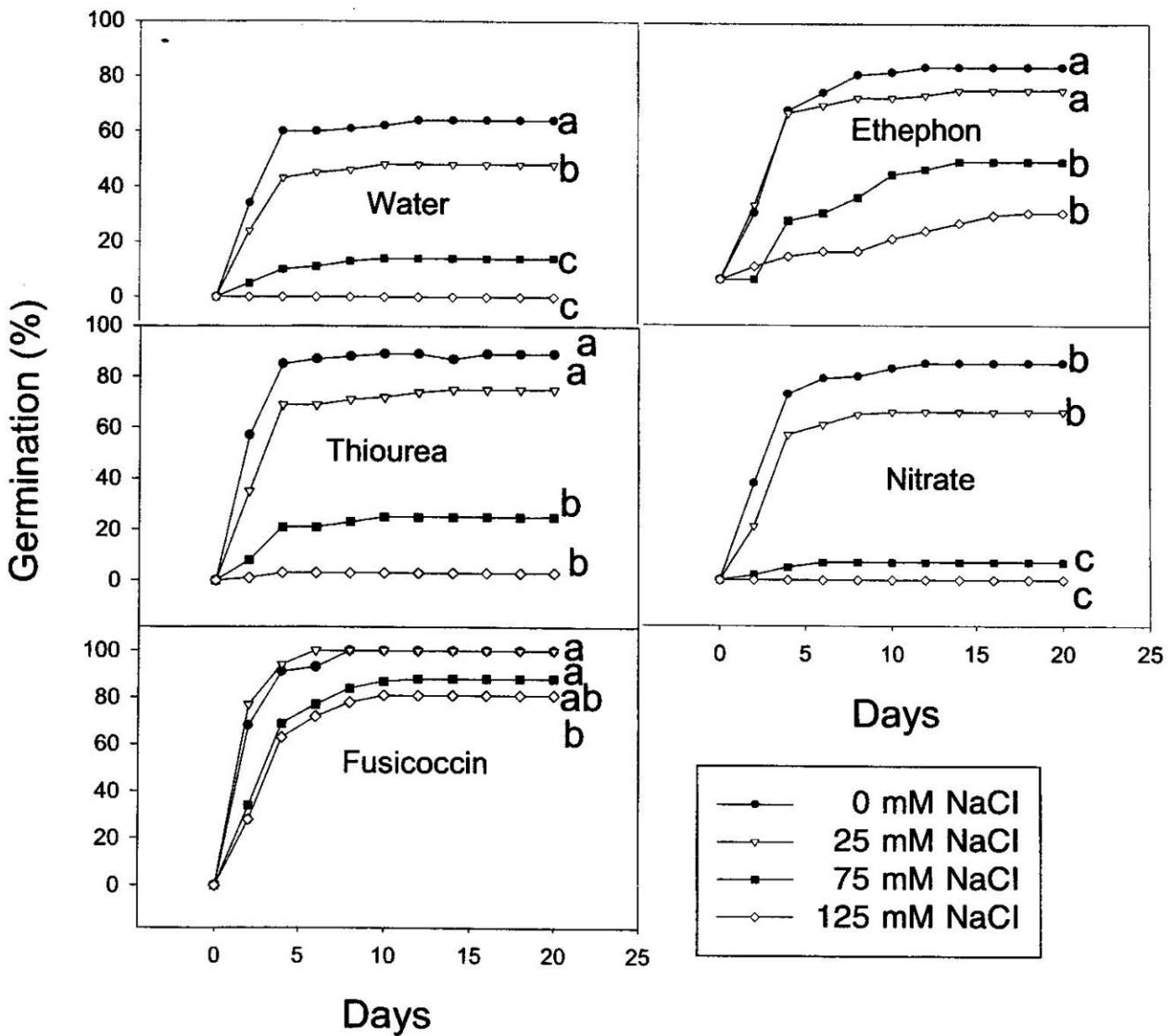


Figure 1. Rate of germination of *Zygophyllum simplex* seeds in 0, 25, 75 and 125 mM NaCl and ethephon (10 mM), thiourea (10 mM), nitrate (20 mM) and fusicoccin (5 μM). Values for each dormancy relieving compound having the same letter are not significantly different ($P > 0.05$).

Table 1. Result of two-way ANOVA of germination by regulators (nitrate, thiourea, ethephon and fusicoccin) and salinity treatments.

Dependent variable	Independent variable		
	Regulators	Salinity (S)	R × S
Germination (%)	108.0***	210.8***	9.16***
Germination (velocity)	135.2***	288.6***	9.28***

Note: Numbers represent F values: *** = $P < 0.0001$.

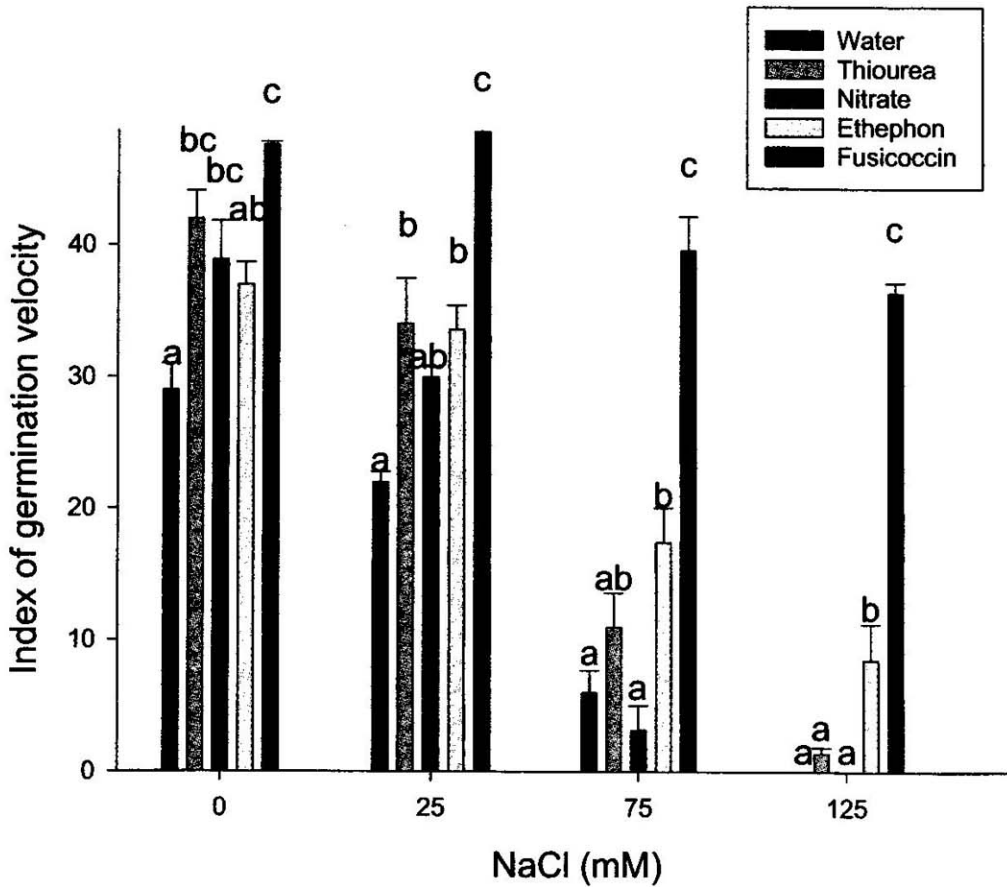


Figure 2. Index of germination velocity of *Zygophyllum simplex* seeds in 0, 25, 75 and 125 mM NaCl and ethephon (10 mM), thiourea (10 mM), nitrate (20 mM) and fusicoccin (5 μ M). Values for each salinity concentration having the same letter are not significantly different ($P > 0.05$).

Discussion

Seeds of *Zygophyllum simplex* have an innate dormancy. Only 60% of the seeds germinated in the non-saline control and germination was also inhibited when salinity was increased. All of the dormancy regulating compounds, thiourea, nitrate, ethylene and fusicoccin, overcame the innate dormancy and stimulated germination of *Z. simplex* seeds in the distilled water controls. The stimulatory effect of nitrates and other simple nitrogenous compounds (thiourea, ammonium salts, nitrites, hydroxylamine) on seed germination of numerous weedy plant species is documented (Roberts and Smith, 1977; Khan and Ungar, 1995; Karssen and

Hilhorst, 1992). Noor and Khan (1995) reported that thiourea alleviated the inhibitory effects of salinity in summer seeds of *Halopyrum mucronatum* at a 10:25°C temperature regime, but at all other temperature and salinity treatments thiourea either had no effect or inhibited germination in comparison to controls. Our data indicates that seed germination of *Z. simplex* is significantly improved with the addition of both nitrate and thiourea separately but they failed to overcome the inhibitory effect of higher salinities on germination.

Germination of *Z. simplex* seeds in distilled water control treatments was stimulated by ethylene. Ethylene plays an essential role in breaking dormancy and stimulating the germination of seeds (Ketring and Morgan, 1972; Corbineau *et al.*, 1990; Smits *et al.*, 1995). Inhibition of germination by salinity in *Z. simplex* is partially alleviated by ethephon. Ethylene also overcame the inhibition of germination imposed by osmotic agents in seeds of *Amaranthus caudatus* L. (Kepczynski and Karssen, 1985) and *Lactuca sativa* L. (Negm and Smith, 1978). However, Ismail (1990) reported that ethylene did not break the innate dormancy of seeds collected from either glycophytic or halophytic populations of *Zygophyllum qatarensis* Hadidi.

Germination of *Z. simplex* in distilled water controls was stimulated by fusicoccin. Ismail (1990) also found that the application of fusicoccin stimulated seed germination of *Zygophyllum qataransis* from both glycophytic and halophytic populations. Fusicoccin is reported to reverse temperature-induced dormancy by increasing K⁺ uptake in germinating seeds (Marre, 1979; Aldasaro *et al.*, 1981). Potassium is a major component of the apoplast and stimulates proton extrusion and photosynthate unloading (Clifford *et al.*, 1986). Salinity induced inhibition of germination in *Z. simplex* is completely reversed by fusicoccin.

Seed germination is the most important process for maintaining populations of the annual *Z. simplex*. Seeds of *Z. simplex* seeds can only germinate when temperature and salinity are reduced (Khan and Ungar, 1997). Monsoon rains not only decrease the salinity of the soil but also substantially reduce the temperature, providing an ideal condition for germination of seeds under field conditions. Khan and Ungar (1997) determined that both proline and betaine alleviated the innate dormancy of *Z. simplex* seeds. Application of fusicoccin, ethylene, thiourea and nitrate also overcame dormancy of seeds in our distilled water controls. Dormancy of *Z. simplex* seeds may be caused by changes in the balance of growth regulator activity due to the presence of a high concentration of ions in its seeds (Khan and Ungar, 1996). Hypersalinity could induce osmotic stress that may result in an increase in inhibitor substances, which disrupts the hormonal balance in seeds and inhibits germination. Of the four germination regulating substances investigated fusicoccin completely alleviated the inhibitory effects of salinity on *Z. simplex* seeds while ethylene only partially promoted germination at higher salinities.

Acknowledgements

M.A. Khan would like to thank CIES, Washington for a Fulbright Scholar Research Grant, Department of Environmental and Plant Biology, Ohio University for the provision of facilities and the University of Karachi for granting a leave. This research was supported in part by National Science Foundation research grant INT-9730882.

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